iGEM 2016 – Microbiology – BMB – SDU

Project type: Silk MaSp1 CA **Project title:** Cloning composite part into iGEM standard plasmid psB1C3. **Sub project:** Insertion of K-1763012 (MaSp1 CA) into pSB1C3 plasmid	**Creation date:** 2016.09.21 **Written by:** Rune Øbo **Performed by:** Rune Øbo, Nete Sloth Bækgaard, Mathilde Nygaard, Rikke Friis Bentzon & Viktor Swaglord Mebus.

1. SOPs in use.

SOP number: SOP0007_v01 LA plates with antibiotic

SOP number: SOP0022_v01 Competent cell - freeze-stock

SOP number: SOP0023_v01 Ca^{++} transformation

SOP number: SOP0009_v01 TSB transformation

Plasmid purification kit

SOP number: SOP0001_v01 ON culture of *E.coli*

SOP number: SOP0004_v01 Bacterial freeze stock

SOP number: SOP0017_v01 Fast digest

SOP number: SOP0015_v01 Ligation

Gel purification kit

SOP number: SOP0021_v01 Colony PCR with MyTaq

2. Purpose.

To insert basic part: K1763012 (MaSp1 CA), into iGEM standard plasmid; pSB1C3.

3. Overview.

Day	SOPs	Experiments
1	SOP0023_v01	Ca^{++} transformation
2	SOP0001_v01	ON culture of *E.coli*
3	Miniprep kit	Plasmid purification
3	SOP0001_v01	ON culture of *E.coli*
4	SOP0004_v01	Bacterial freeze stock
5	SOP0017_v01	Fast digest
5	Gel purification kit	Gel purification
6	SOP0015_v01	Ligation
6	SOP0009_v01	TSB Transformation
7	SOP0021_v01	Colony PCR with MyTaq
7	SOP0001_v01	ON culture of *E.coli*
8	Miniprep kit	Plasmid purification
8	SOP0017_v01	Fast Digest with EcoRI + PstI
9	SOP0004_v01	Bacterial freeze stock of ON.

4. Materials required.

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Appropriate medium ex. LB	1% Tryptone 1% NaCl 0.5% Yeast extract	Oxoid Sigma-Aldrich Merck	Media lab or V18-40 5-0	
Glycerol	50 %	AppliChem	Anne Mette, RT	
LB		Anne-Mette		

LA	1% Tryptone 1% NaCl 0.5% Yeast extract 1.5% agar	Oxoid Sigma-Aldrich Merck Difco agar from BD	Anne-Mette Or V18-405-0	
Water	Demineralised milli-Q autoclaved water	Milli-Q water purification system (Millipore)	RT	Water
MyTaq™HS Red Mix	http://www.bioline.com/documents/product_inserts/MyTaq%E2%84%A2%20HS%20Red%20Mix.pdf#zoom=130	Bioline	V18-405a-2	
Reverse primer	Made specific to the template	Sigma-Aldrich		
Forward primer	Made specific to the template	Sigma-Aldrich		
Ligasebuffer		Agilent Technologies	Freezer at 1. Floor	
Ligase			Freezer 1. Floor	Ligase
FastDigest enzyme		Agilent Technologies	Freezer at 1. Floor	EcoRI PstI
Fast digest green / 10 x FastDigest Buffer		Agilent Technologies	Freezer at 1.	
CaCl,	0.1M		Chem room	
MgCl,	0.1M		Chem room	MgCl,
liquid nitrogen	liquid nitrogen	liquid nitrogen	liquid nitrogen	
6x DNA Loading Dye		GeneRuler	fridge floor 1	
Fort. LB		The new Anne-Mette	Autoclave room	
Magnesiumchloride (MgCl2) 1M	1M	The New Anne-Mette	Autoclave	

Polyethylene glycol (PEG) 3.350	Sigma Aldrich	Micro Chemical room
Dimethylsulfoxid (DMSO)	Sigma Aldrich	Micro Chemical room

5. Other

As competent cells, LB and LA media was used by all parts of our project and not just this protocol the dates for use of these SOPs are not added. this comment deal with SOP number: SOP0007_v01 and SOP0022_v01

Gel Electrophoresis is set at 75 V for 30-45 minutes, dependent on the gel percent.

6. Experiment history.

Date (YY.MM.DD)	**SOPs**	**Alterations to SOPs and remarks to experiments**
16.06.27		Resuspend the received 200ng gblock from IDT with dH2O. Thereafter take 5µl of dulited gblock and add 95µl dH2O making a 20X dilution. (K1763012) Name: SG5
16.07.22	SOP0017_v01 Fast Digest	Here we used the Bacto-groups plasmid with inserted bactoricine, lysostaphin. Fast Digest with EcoRI for 30min and PstI for 2h. Gel electrophoresis.
16.07.22	Gel Purification kit.	Ran the gel electrophoresis for 40min 75V. Cut out and purified the backbone psB1C3. Name SG58.
16.07.22	SOP0010_v01 Phusion PCR	Phusion PCR of SG5. Program used for PCR:

Temp (ºC)	Time (min)
95ºC	3:00
95ºC	0:25

		58ºC | 0:25 72ºC | 0:20 Repeats | x34 72ºC | 1:00 20ºC | Infinite . Name: SY9
16.07.22	SOP0017_v01 Fast Digest	Fast Digest of SY9 with PstI for 2h at 37ºC and EcoRI for 0.5h at 37ºC. After digestion the enzymes were denaturated at 80ºC in 10min. Name: SG45
16.07.22	SOP0015_v01 Ligation	Ligation of SG58 (digested backbone) and SG45 (digested gblock). Ligation took place at 16ºC for 16h. Name: SB67

Ratio	1:2	1:5
Ligase Buffer	2µl	2µl
Ligase	1µl	1µl
SG58	1µl	1µl
SG45	2µl	5µl
H2O	→20µl	→20µl

16.07.23	Enzyme inactivation	Denaturation of enzyme ligase with 65ºC for 10min.
16.07.23	SOP0009_v01 TSB transformation	16.07.22 SOP0001_v01 ON culture of *E. coli.* Top 10. OD about 0.3-0.5nm. Thereafter a regular TSB transformation with SB67. Plating after TSB transformation.
16.07.24	SOP0021_v01 Colony PCR with MyTaq	Taking 3 colonies from the plate to increase the odds of one colony to be correct. Primers VR and VF2. Using the same pipet tip, the colonies were also set to an ON. Half of the colony PCR product were added EcoRI and PstI to see if they were able to recognize. Gene around 119bp.

Temp	Time (min)

		95ºC	3:00
		95ºC	0:25
		58ºC	0:25
		72ºC	0:30
		Repeats	34X
		72ºC	1:00
		20ºC	Infinite
16.07.24	Gel electrophoresis	2% agarose gel, 75V for 30min.	
16.07.25	SOP0004_v01 Bacterial Freeze stock	900µl of the previous ON culture were added into 125µl glycerol and put in the -80ºC freezer. Name: #39	
16.07.25	Miniprep Kit (plasmid purification kit)	Plasmid purification on the same ON culture as freeze stock. Name: SR37.	
16.07.26	Sequencing	Sent SR37 to sequencing.	

7. Sample specification.

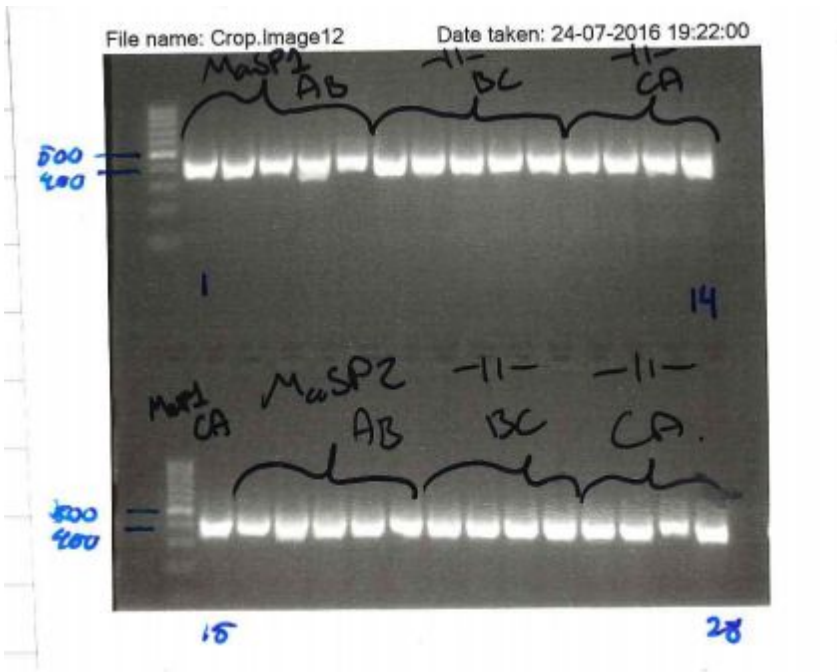
Sample name	Sample content	From	Used for / Saved where
SG5	MaSp1 CA g block: K1763012	IDT	Diluted with dH2O / saved in coolbox
SY9	Phusion PCR product of SG5	IDT	Used to cut with restriction enzymes EcoRI and PstI.
SG58	purified psB1C3	iGEM	Vector cut with pst1 and EcoR1 / cool box
SG45	Fast digest of SY9		Cut with PstI and EcoR1 / Saved in coolbox

SB67	pSB1C3 ligated with SG45	vector and basic part ligated and transferred to *E.coli.* /Saved in coolbox
SR37	pSB1C3:k176312 (MaSp1 CA)	purified and transferred SB67/ freeze stock in *E.coli* (#39)

8. Remarks on setup.

9. Results and conclusions.

The first gel photo below shows the colony PCR results. The second gel photo shows cutting of SB75 with Pst1 at 2 hours with 37 ºC and EcoR1 at 30 minutes, and after that called SR40. Every second well had added restriction enzymes. The composite part is 119bp. It is possible from the Gel photo to assume that the DNA is cut correctly. VR and VF2 primers adds a total of 300bp, so a total of around 419bp. From this gel the ligation and transformation of SB75, band with around 119bp after digestion, were concluded successfully and sent to sequencing.



Picture of colony PCR of different colonies on the same plate, but each gene had its own plate. All the colony PCR shows a band near the 500makr which was desired.

This gel photo shows the cutting of either PstI and also another cut with PstI and EcoRI. Every second well had added two restriction enzymes. The markings in between the 200 mark and 100mark og base pairs indicates our correct gene. The first 6 wells are MaSp1 and the last 6 are the genes for MaSp2. Ladders were added on both sides, blue and green.

10. Appendixes